Assessment of Genetic Diversity and Adaptability of Inbred Lines and their F_1- hybrids of Grain Maize (*Zea mays* L.), Using Molecular Markers (RAPD) and AMMI Analysis

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ABSTRACT

This study was carried out to assess the genetic diversity and adaptability of seven inbred lines of grain maize (*Zea mays* L.) and their F_1-hybrids, using molecular markers (RAPD) and Additive Main Effects and Multiplicative Interaction (AMMI), respectively. A field experiment was carried out during the winter and summer of 2009 and 2010 at two locations, Shambat and Elrawakeeb. A split-plot design with three replications was used to layout the experiment. The inbred lines and their F_1-hybrids were field-evaluated for grain yield/ha under normal irrigation and water-stress conditions. The DNA molecular markers (RAPD) analysis showed that high level of polymorphism of 89.33 percent was detected among the genotypes, which were distinguished into four main groups (sub-clusters). The genetic distance among inbred lines ranged from 0.05 to 0.33. The inbred line 66y was the most distant line compared to other inbred lines; it represents a single group in the cluster. The inbred lines 66y and 160 had the greatest genetic distance of 0.31. AMMI analysis differentiated the genotypes (inbred lines and F_1-hybrids), based on their interaction to different environments, into diversified adaptation pattern. The hybrid 160x66y showed the highest (4.1 tons/ha) grain yield (highest heterosis) and a moderate positive interaction (PCA score = 19.0), indicating its adaptability to favorable environments. Moreover, the hybrid 66y×3 showed considerable yield (2.4 ton/ha) and adaptation to water stress environments. These results revealed that crossing of the most genetically-distant inbred lines (e.g., hybrid 160x66y) gave the highest heterosis, which could be utilized for improvement of grain yield of maize. Also, it could be concluded that DNA markers were efficient in the assessment of genetic diversity to identify the most appropriate inbred lines of maize for development of hybrid varieties, and then avoiding crosses between genetically-related inbred lines. Also AMMI analysis was successful to quantify the interaction and adaptability of the tested genotypes to wide range of environments.

Key words: Maize; AMMI model; adaptability; molecular markers; RAPD

INTRODUCTION

Maize (*Zea mays* L.) (2n=20), which is also known as corn, belongs to the family Poceaceae. Maize is the third most important crop worldwide (Frava *et al.,* 1999). Maize grows over wider geographical and environmental ranges than any other cereal crop. It is grown at latitudes varying from the Equator to slightly Northern and Southern of latitude 50°, from sea level to over 3000 meters elevation under heavy rainfall and semi – arid conditions, cool and very hot climates. About half of the world maize area is located in developing countries where maize flour is a staple food for poor people and maize stalks provide dry season feed for farm animals (Ahmed, 2011). Diversified uses of maize worldwide include grain, starch products, corn oil and forage for animals (Abdelmula and Sabiel 2007).

In Sudan, although maize is of less importance than sorghum, wheat and millet as a staple human food, the crop plays a great role in food security for the people in Blue Nile and South Kordofan States (Ahmed and Elhag 1999). The crop is grown in the two states by traditional farmers in small-holdings under rain. Nowadays, different companies and individuals started to grow the crop on a large scale under irrigation or under rain in different parts of Sudan. However, the total cultivated area of maize in the Sudan increased from 17 thousand hectares in 1971 to 37 thousand hectares in 2010 (Ahmed 2011). The average grain yield of maize (109 kg/ha) is far below that of the world (6 t/ ha) (AOAD 2007). The low productivity of maize was attributed to the low yield stability of the local open-pollinated cultivars that are normally grown and to the greater sensitivity of the crop to water stress (Saliem, 1991).

Selection based on yield only, may not always be adequate when genotype by environment interaction is significant (Kang and Pham, 1991). The presence of genotype by environment interaction (GEI) frequently changes the hybrid ranks in different environments due to cross interaction making their proper selection difficult. Therefore, it is essential that the genotype by environment interaction is taken into account, properly understood and analyzed. However, analysis of interaction of genotypes with locations and other agro-ecological conditions would help in getting information on adaptability and stability of performance of genotypes. The method commonly used for analysis of G×E interaction is the Linear Regression model of Eberhart and Russell (1966) in which the *bi*-values give information about adaptability and S^2d are used as measures of stability of performance. Other workers (e.g. Zobel *et al.,* 1988) suggested the use of AMMI (Additive Main and Multiplicative Interaction) approach as a measure of stability and adaptability. The AMMI model is a better model for analysis of GxE interaction in multiplication varietal trials (Zobel *et al.,* 1988). It does not only give estimate of the total GxE interaction effect of each genotype but also partitions it into interaction effects due to environments.

The use of DNA-based molecular markers for the genetic analysis and

manipulation of important agronomic traits has become an increasingly useful tool in plant breeding. However, the genetic diversity evaluation by the means of the molecular markers presents some advantages over other methods because, in addition to identifying the high polymorphism, they do not present interactions with the environment, and can be evaluated at any stage of development (Williams *et al.,* 1990). Among the different types of molecular markers, randomly amplified polymorphic DNAs (RAPDs) are useful for the assessment of genetic diversity (Williams *et al*., 1990) because of their simplicity, speed and relatively low-cost (Rafalski and Tingey, 1993) as compared to other types of molecular markers. RAPD can be used in studying genetic diversity, phylogeny, quantitative trait loci and varietal identification (Weising *et al*., 1995). In maize, this technique has been widely used in diversity studies because, in addition to its low cost, it allows polymorphism to be detected in a simple and fast manner (Liu *et al*., 1998; Wu, 2000). Therefore, the objective of this study was to analyze G×E interaction and evaluate the adaptability of grain maize genotypes, using AMMI analysis and to estimate genetic diversity among inbred lines, using molecular markers (RAPD), and its relationship to grain yield adaptability among F1-hybrids.

MATERIALS AND METHODS

Plant material and Experimental details

Plant material used in this study consisted of seven parental inbred lines (66y, 160, 3, 2, 405, 277 and 6), thirteen F_1- hybrids and two standard commercial cultivars (Huediba-1 and Huediba-11) of maize (*Zea mays* L.) (Table.1). The first Field experiments were carried out during the winter and summer seasons of the two years 2009 and 2010 at the Experimental Farm of the Faculty of Agriculture, University of Khartoum at Shambat (32º:32´E. Longitude, 15º:40´N. Latitude and 380 meters above the sea level). The second field experiment (summer 2010) was carried out at Elrawakeeb Dry lands and Desertification Research Station, National Centre for Research, about 35 Km west of Khartoum (32º:15´ E. Longitude, 15º:25´ N. Latitude and 420 meters above the sea level). The genotypes were evaluated under two levels of water treatment; namely normal irrigation every 7 days and water stress by irrigating every 21 days, and under four different environments namely: [Shambat winter season 2009 (SW09), Elrawakeeb summer season 2010 (ERS10), Shambat summer season 2010 (SS10) and Shambat winter 2010 (SW10)]. A split- plot design with three replications was used to execute these experiments. The water treatments were assigned to the main-plots and genotypes to the sub- plots. Each genotype was grown in a 4×5 meters/plot at a seed rate of 3 – 4 seeds/hill on ridges during the last week of July for summer season and the first week of November for winter season. Thinning was carried out after a week from sowing, to raise two plants/ hill. Hill-to-hill and ridge-to-ridge spacing was 20cm and 70 cm, respectively. Agronomic and cultural practices, i.e., fertilizer application, weeding, irrigation and plant protection procedures were adopted when required according to recommendations. The

grain yield (kg/ha) was calculated for each genotype, under each environment.

Table 1. List of maize genotypes studied and their types

Genotype code	Genotype	Type
1	66y	Inbred Line
2	277	″
3	3	″
4	6	″
5	2	″
6	160	″
7	405	″
8	Hudieba-1	Improved Open pollinated
9	Hudieba-2	Improved Open pollinated
10	66y× 405	Hybrid
11	66y × 277	Hybrid
12	66y × 6	Hybrid
13	160 × 277	Hybrid
14	160 × 3	Hybrid
15	160 × 66y	Hybrid
16	160 × 6	Hybrid
17	66y × 2	Hybrid
18	405 × 160	Hybrid
19	405 × 6	Hybrid
20	6 × 3	Hybrid
21	2 × 160	Hybrid
22	66y × 3	Hybrid

Statistical analysis

G×E interaction was analyzed using Additive Main Effect and Multiplicative Interaction (AMMI) model (Zobel *et al.,* 1988), to identify adaptation pattern of the different genotypes in the 8 environments.

Molecular assessment of genetic diversity

DNA extraction: Genomic DNA was extracted from fresh leaf tissue of 27 maize genotypes (Table.1), using modified CTAB method (Porebski *et al*., 1997). In this method the fine powdered plant materials were immediately transferred into 13 ml Falcon tubes containing 6 ml of pre-warmed lysis solution. Tubes containing the samples were then incubated in a water path at 65ºC with gentle shaking for 30 min and left to cool at room temperature for 5 min. Isoamyl and chloroform mixture (1:24) was added to each tube and the phases were mixed gently for 5 min at room temperature to make a homogenous mixture. The cell debris was removed by centrifugation at 5000 rpm for 15

min and the resulted clear aqueous phases (containing DNA) were transferred to new sterile tubes. Chloroform: isoamyl alcohol extraction was repeated twice. The nucleic acids in the aqueous phase were precipitated by adding equal volume of deep cooled isopropanol. The contents were mixed gently and collected by centrifugation at 4000 rpm for 10 min. The formed DNA pellet was washed twice with 70% ethanol and the ethanol was discarded after spinning with flash centrifugation. The remained ethanol was removed by leaving the pellet to dry at room temperature.

Data Analysis: For each primer, the number of polymorphic and monomorphic bands was determined. Bands clearly visible in at least one genotype were scored (1) for present, and (0) for absent and entered into a data matrix. Fragment size was estimated by interpolation from the migration distance of marker fragments. Percentage of polymorphism was calculated as the proportion of polymorphic bands over the total number of bands. The genetic dissimilarity (D) matrix among genotypes was estimated according to (Nei and Lei, 1979). The similarity coefficient was used to construct a dendrogram by the un-weighted pair group method with arithmetic averages (UPGMA) according to Rohlf (1993).

RESULTS

AMMI analysis

The AMMI analysis of variance showed significant effect for genotypes, environment and G×E interaction (Table 2). These results showed that , the variation due to environments (E), genotypes (G), and the genotype × environment interaction (G×E) was highly significant ($P<0.01$) and accounted for 41.3, 34.5 and 24.2% of the total sum of squares (ESS+GSS+GEI SS), respectively (Table 2). Large variation among the F_1-hybrids as well as the inbred lines for grain yield (kg/ha) and their interaction to the environments was determined (Table 3). The highest average grain yield (kg/ha) was obtained for the environment $SSD_0$10 (3747 kg/ha) and the lowest (1620 kg/ha) for the environment $ERSD_1$10 (Fig 1). Among the eight environments $SWD_0$09 and $SWD_1$09 exhibited the highest (+42.6 and +29.4) positive PCA scores, respectively, while, $ERSD_1$10 and $SSD_1$10 showed the highest (-32.4 and -10.6) negative PCA scores, respectively (Fig. 1). However, the environment $SWD_0$10 scored the smallest (-2.5) negative interaction (PCA scores). Among the inbred lines, the largest (+5.3) positive score of PCA was shown by line 2 and the largest 2 and the largest (-17.7) negative score was exhibited by inbred line 3 (Fig.1). The F_1-hybrids showed high variability in grain yield (kg/ha) and their interactions (PCA scores) to the different eight macro-environments (Fig. 1). The hybrid 66y×2 exhibited the highest (+46.0) positive interaction (PCA score), whereas, the hybrid 66y×3 showed the highest (-15.1) negative PCA score. However, the hybrid 160×66y has the highest yield among all hybrids and

moderate (+19.0) positive PCA score (Fig.1). The hybrids which showed the smallest interactions (PCA scores) were 160×3, 66y×6 and 6×3 (Fig.1).

Table 2. Sum of squares (SS), mean squares (MS) and variance components (var. comp.) from analysis of variance of 22 maize genotypes (parental lines, F_1- hybrids and check varieties) evaluated in eight macro–environments[1] for grain yield (kg/ha)

Var. comp	d.f	SS	EMS	Var. comp.
Environment[1]	7	231397413.4	1152782.7	
Rep with Env	16	18444523.7	9205690.4	28.7**[2]
Genotypes	21	193319498.1	924110.9	9.96**
Genotypes × Envir	147	135844304.4	450651.79	2.05**
Residual[3]	336	151149000.2		

[1]Eight macro-environments (combination of 2 locations × 2 years × 2 treatments), see materials and methods (ERD_0S10, ERD_1S10, SS $D_0$10, SS $D_1$10, SW $D_0$09, SW $D_1$09, SW $D_0$10 and SW $D_1$10)

[2]*, ** Significant at the 0.05 and 0.001 probability level, respectively

[3]Residual= deviations from regression

Table 3. Variance components of AMMI analysis for 22 genotypes (Parental inbred lines, F_1-hybrids and check varieties): Sum of squares (SS), mean squares (MS) and variance components (Var. comp.) for grain yield (kg/ha), averaged over three replications and across eight macro-environments

Sources	d.f	SS	MS	Var. comp
Total	527	730424739.8		
Environment	7	231397413.4	33056773.3	28.68**
Rep with in Env.	16	18444523.7	1152782.7	
Genotypes	21	193319498.1	9205690.4	9.96**
Genotypes × Env.	147	135844304.4	924110.9	2.05**
PCA 1	27	50281927.4	1862293.6	4.13**
PCA 2	25	35956537.1	1438261.5	3.19**
PCA 3	23	16304076.7	708872.9	1.57*
PCA 4	21	12871581.1	612932.4	1.36ns
PCA 5	19	11513085.0	605951.8	1.34ns
PCA 6	17	4935981.1	290251.8	0.64ns
PCA 7	15	3981116.0	265407.7	0.59ns
Residual	336	151419000.180	450651.79	
Grand mean = 2622		R- squared = 0.79		C.V= 25.60%

[1]Eight macro-environments (combination of 2 locations × 2 years × 2 treatments), see materials and methods (SW $D_0$09, SW $D_1$09, SS $D_0$10, SS $D_1$10, ERD$_0$S10, ERD$_1$S10, SW $D_0$10 and SW $D_1$10).

[2]Residual= deviations from regression

.*, ** Significant at the 0.05 and 0.001 probability level, respectively; ns. Non-significant; PCA: Principal Component Analysis

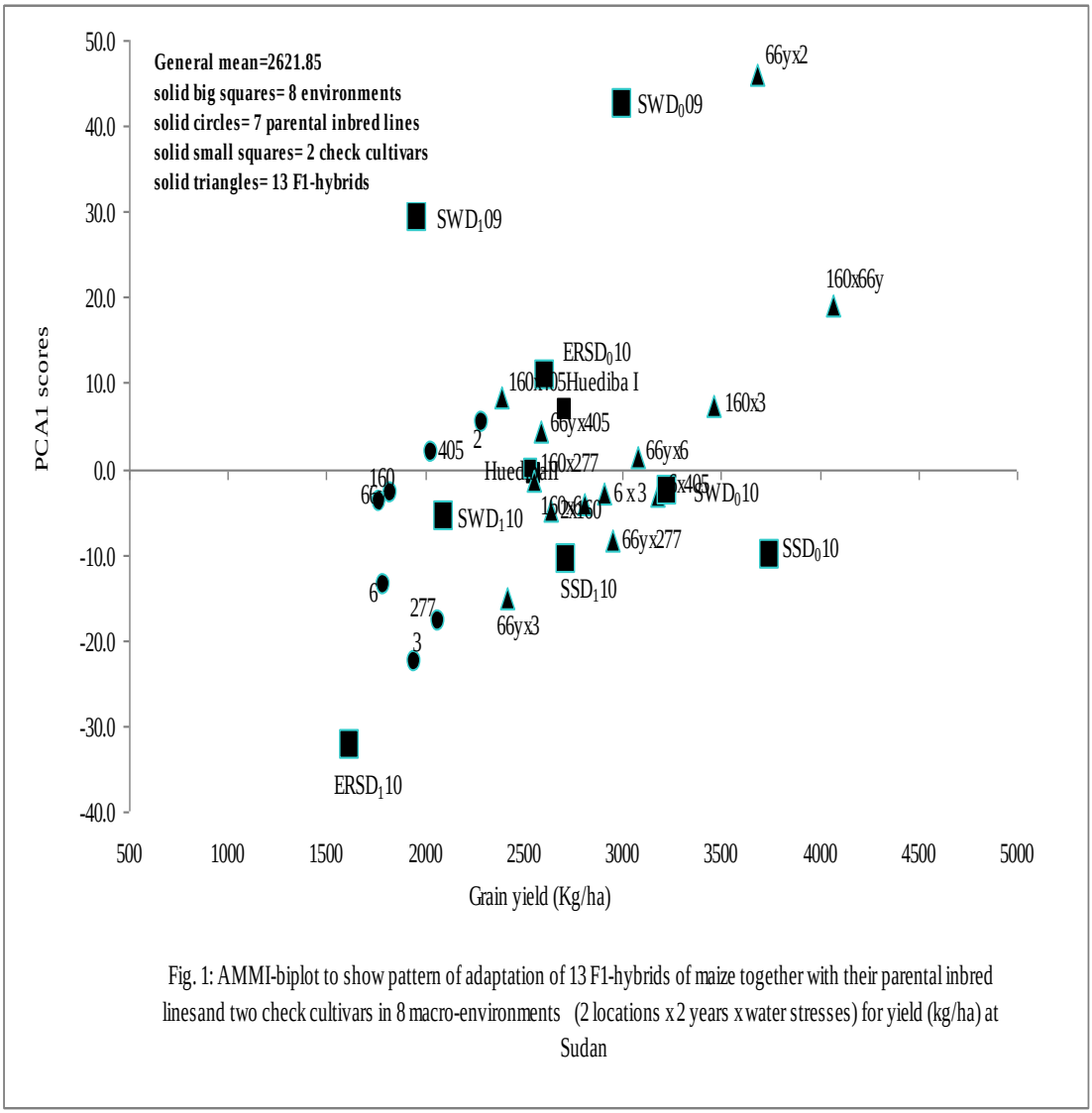


Fig. 1: AMMI-biplot to show pattern of adaptation of 13 F1-hybrids of maize together with their parental inbred linesand two check cultivars in 8 macro-environments (2 locations x 2 years x water stresses) for yield (kg/ha) at Sudan

Genetic diversity among maize genotypes using RAPD marker

Seventeen random primers were used to asses' genetic diversity among 27 maize genotypes, of which 10 were observed to be polymorphic.10 polymorphic primers along their sequence, are shown in (Table 4). Total of 59 amplified fragments were distinguished across the selected primers and statistical analysis showed polymorphic bands among the genotypes with average of polymorphic bands per primer. The maximum numbers of fragment bands were produced by primers A_1 and C_2 (10 bands) with (100%) polymorphism, while the minimum number of fragments were produced by primer OPA_{20} (Fig. 2).

Cluster analysis using average linkage methods (UPGMA) distinguished three basic groups (Fig.3). One of these main clusters divided into four sub-clusters including 23 genotypes. One of these four sub-clusters is represented by only one genotype (66y). The most genetically related parental lines included in these four sub-clusters were 6 and

160, while the most genetically far distant parental lines were 66y and 160 (Fig.3). Each of the two other main clusters composed of two distinct genotypes, one main cluster is represented by the hybrids 66y×277 and 3×2, and the other by the hybrids 2×277 and 2×6 (Fig.3). The most genetically related hybrids observed were 160×2, 160×6, 66y×3 and 66y×6, while the most genetically far distant hybrids were 66y×3 and 2×6 (Fig.3).

Correlation between genetic distance detected by RAPD marker and adaptability of inbred lines and F_1-hybrids using AMMI analysis were positive and significant. However, the highest (4.1 tons/ha) grain yield, a moderate positive interaction and adaptability to favorable environments were recorded for 66y×160 combination. This association of groups showed genetic distance of 0.31. Moreover, the highest grain yield (2.4 ton/ha) and adaptation to water stress environments was observed between 66y × 3 combination with genetic distance 0.28. Such results were excepted since combinations between similar heterotic groups could only present genetic divergence grain yield and adaptability lower than the combination between distinct groups.

Table 4. Polymorphism detected by the use of 10 random primers on 27 maize (*Zea mays* L) genotypes

Name of primer	Sequence of primer (5′- 3′)	Total No. of polymorphic bands	No. of polymorphic bands	Percentage of polymorphic bands
A_1	CAGGCCCTTC	10	10	100
B_7	GGTGACGCAG	5	4	80
C_2	GTGAGGCGTC	10	9	90
C_8	TGGACCGGTG	5	5	100
D_{20}	ACCCGGTCAC	5	4	80
OPA_{17}	GACCGCTTGT	5	5	100
OPA_{20}	GTTGCGATCC	3	3	100
UBC_{101}	GCGGCTGGAG	5	54	80
UBC_{106}	CGTCTGCCCG	6	4	83.3
UBC_{155}	CTGGCGGCTG	5	4	80
Total		59	53	893.3
Average		5.6	5.3	89.33

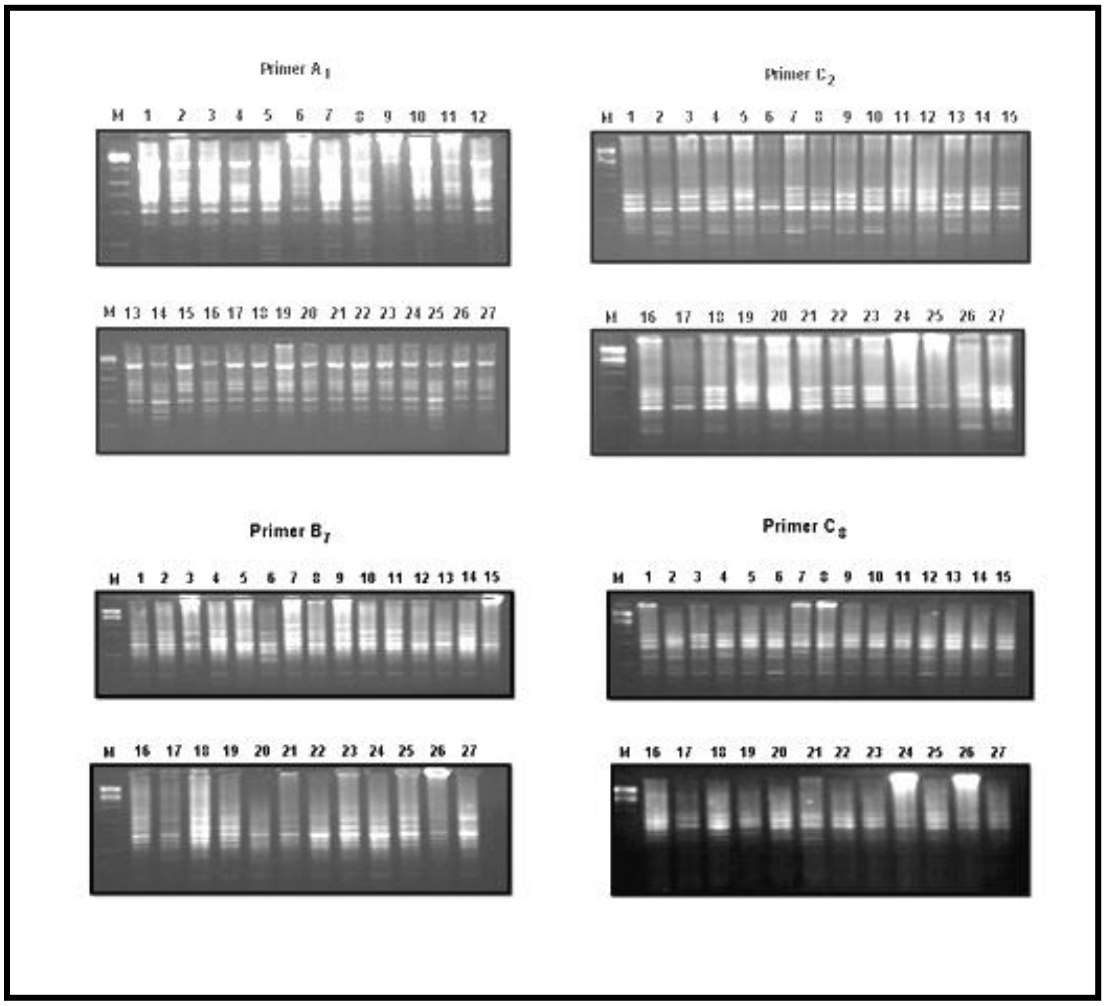
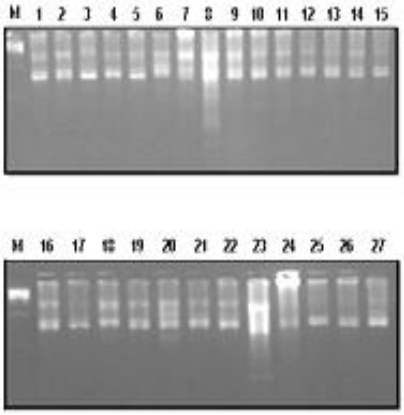


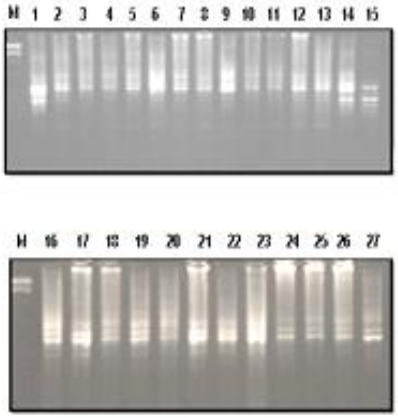
Fig. 2. RAPD amplification patterns with primers A_1, C_2, B_7, C_8, OPA_{17}, UBC_{101}, UBC_{155}, OPA_{20}, UBC_{106} and D_{20} (from left to right. Genotypes1-27 and M-Ladder 1Kb).

Fig. 2 (contin)

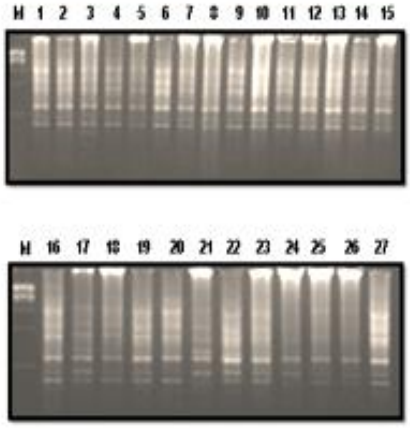
Primer OPA_{17}



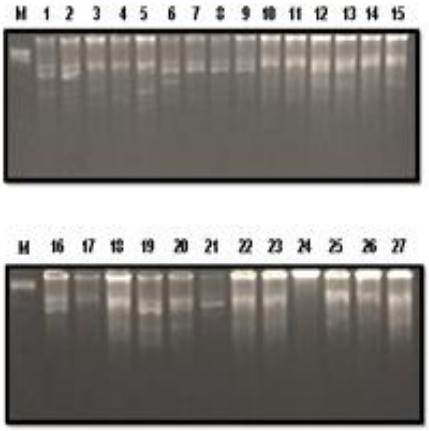
Primer UBC_{101}



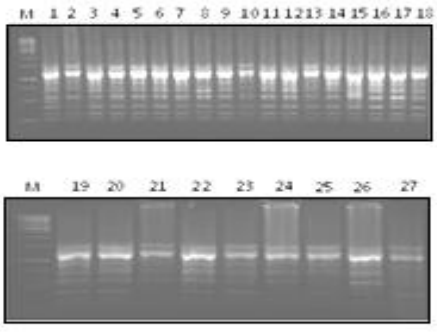
Primer UBC_{155}



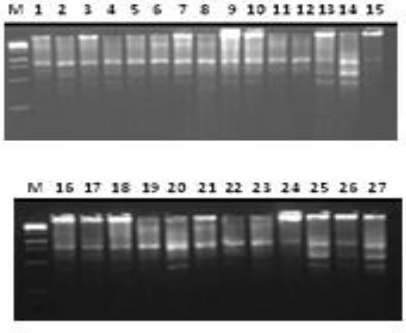
Primer OPA_{20}



D_{20} Primer



UBC_{106}



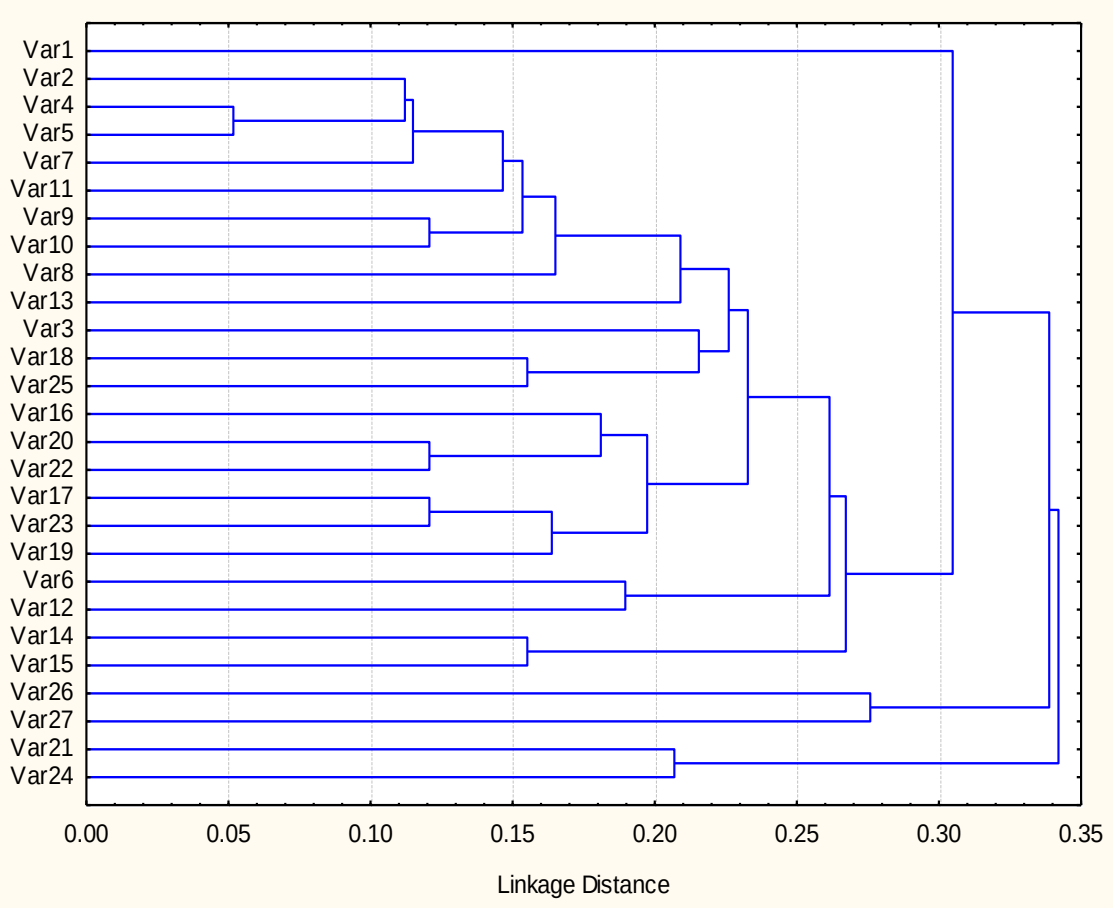


Fig. 3: Dendrogram constructed for 27 maize (*Zea mays* L.) genotypes based on genetic distances using 10 RAPD Primers

Key

Var1	66y	Var10	6×3	Var19	405×277
Var2	277	Var11	277×6	Var20	66y×3
Var3	3	Var12	66y×405	Var21	2×277
Var4	6	Var13	66y×277	Var22	66y×6
Var5	160	Var14	3×405	Var23	160×6
Var6	2	Var15	66y×160	Var24	6×2
Var7	405	Var16	160×277	Var25	160×3
Var8	Huediba-1	Var17	160×2	Var26	66y×277
Var9	Huediba-11	Var18	160×405	Var27	3×2

DISCUSSION

Adaptation of inbred lines and F_1- hybrids

The AMMI analysis of variance for grain yield (kg/ha) of the 22 genotypes showed that 41.5% of the total variation was attributable to environmental effects, only 35.5% to genotypic effect and 24.2% to genotype × environment interaction effect. A large variance due to environments indicated that the environments were diverse, with large differences resulted in most of the variation in grain yield (kg/ha). The magnitude of the genotypic effect (G) was larger than that for genotypes × environments interaction (GEI), indicating that there were substantial differences in genotypic response across environments. Similar findings were reported by Nobel *et al*. (1988) and Crossa *et al*. (1997). Results from AMMI analysis also showed that the first principal component axis (PCA1) of the interaction was adequate to differentiate between the genotypes (inbred lines and F_1-hybrids) according to their adaptability. For example, the AMMI analysis showed that the most productive (3747 kg/ha) environment was $SSD_0$10 and the less productive (1620 kg/ha) one was $ERSD_1$10. On the other hand, environment $SWD_0$09 showed the largest interaction score (+42.6) and environment $SWD_0$10 showed the smallest interaction score (-2.5). This result indicates that Shambat winter season under normal irrigation ($SWD_0$10) has less effect on genotypes performance, compared to other environments, whereas environment $ERSD_1$10 exhibited the highest (-32.4) negative interaction score, indicating its high drought severity resulting in great reduction of the tested genotypes.

Based on mean performance (grain yield kg/ha), and according to AMMI biplot, the inbred lines and the different hybrids exhibited different pattern of adaptations. For example, the hybrid 66y×2 showed the highest positive interaction and more adapted to the favorable environment $SWD_0$09, and $ERSD_0$10. The hybrids 160 x 66y showed the highest yield and moderate positive interaction, indicating its stability and adaptation also to the most productive favorable environments ($SWD_0$09 and $ERSD_0$10). However, the hybrids 66y×6, 160×2, 405×6 and 6×3 exhibited an interaction (PCA scores) closer to zero, indicating their high yield stability. Moreover, the hybrid 66y×3 showed considerable yield and adaptation to drought environments (e.g., $SWD_1$10 and $ERSD_1$10). Based on AMMI analysis, generally, the inbred lines had inherited their adaptation as well as yield performance to their F_1-hybrids, like inbred line 3 and 277, which produced hybrids (66y×3 and 66y×277) with negative scores and low yields. Similar results were reported by Ajibade *et al.* (2002) and Abera *et al.* (2004), who analyzed the genotype-environment interactions and phenotypic stability of maize.

Molecular markers and genetic diversity

Knowledge on the genetic diversity and relationships among maize inbred lines is indispensable to identify promising combinations for exploitation of heterosis and

establishment of heterotic groups for use as source materials in a breeding program. In this study, large amount of genetic diversity (89.33%) among genotypes was revealed by selected primers. The estimated diversity in this study was higher than in some previous maize studies, such as reported by Melo *et al.* (2001), who obtained 61.46% of polymorphic bands working with hybrids and Lanza *et al*. (1997), who obtained 80.6% of polymorphism, studying genetic divergence between inbred lines using RAPD markers.

The extent of genetic variation in 27 maize genotypes was characterized based on dissimilarity matrix by UPGMA dendrogram which divided the genotypes into three major clusters. One of these main clusters divided into four sub-clusters including 23 genotypes. One of these four sub-clusters is represented by only one genotype (66y). These results indicated that, high genetic diversity among parental lines used in this study. The most genetically related parental lines included in these four sub-clusters were 6 and 160, while the most genetically far distant parental lines were 66y and 160. On the other hand, clustering of hybrids based on genetic diversity showed good agreement with their pedigree data, because hybrids with similar parental components were joined together in smaller group e.g. the hybrids 160×2 and 160×6 as related, the hybrids 160×405 and 160×3 as related and the hybrid 2×277 and 6×2 as related which were genetically distant from all other genotypes. These results are in agreement with the heterotic patterns described by Lanza *et al*. (1997) who described that RAPD markers are useful to establish consistent heterotic groups between corn lines.

Genetic diversity and adaptability among different environments

In the current study, highly significant positive correlations manifested between RAPD marker-based genetic distance and adaptability of hybrid among different environments, indicating the effectiveness of molecular markers for prediction of hybrid performance. However, unrelated parental lines (lines 160 and line 66y) obtained the highest grain yield (kg/ha) among all hybrids and moderate positive PCA score. These results are in agreement with results obtained by Boppenmeier *et al.* (1992) and Melchinger (1993), who described that molecular DNA markers have been used to analyze the genetic relationships among maize inbred lines and to examine the relationship between DNA marker-based genetic distance and single-cross grain yields in maize genotypes. Generally, the information of RAPD markers for diversity analysis can be used for better understanding of the genetic relationships among the inbred lines, more effective utilization of the inbred lines in the breeding programs for the development of varieties, and formation of heterotic populations used to derive promising inbred lines.

CONCLUSION

It can be concluded that DNA markers are efficient in the assessment of genetic diversity to identify the most appropriate inbred lines of maize for development of hybrid

varieties, and thus avoiding crosses between genetically-related inbred lines. Also AMMI analysis was successful to quantify the interaction and adaptability of the tested genotypes to a wide range of environments.

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